

Original article

The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair®) treatment

Background: Some patients with allergic asthma treated with anti-IgE (Xolair®) do not become symptom free. Better criteria for response assessment than allergy skin tests or IgE determination are needed. The impact of the size of the disease relevant allergen-specific IgE antibody fraction, i.e. the percentage of IgE antibody of total IgE, was evaluated in cat allergic patients treated with the recommended doses of Xolair®. Results were measured as changes in basophil allergen threshold sensitivity (CD-sens).

Methods: In a double-blind placebo controlled trial 20 patients with a high (>3.8%) and 18 with a low (<1%) percentage of IgE antibodies to cat were given Xolair® for 16 weeks and the change in CD-sens was compared to 11 and 10 patients, respectively, in each group receiving placebo.

Results: The CD-sens dropped significantly in both the high ($P < 0.001$) and low ($P < 0.001$) group on Xolair® but did not change significantly after placebo. For Xolair®-treated patients, at the end of the trial there was a highly significant ($P < 0.001$) difference in CD-sens between the high group, where no patients, and the low group, where 13/18 patients, had become negative.

Conclusions: The currently recommended doses of Xolair® very efficiently eliminate IgE antibodies if the IgE antibody fraction is <1% of total IgE but has not enough effect on allergen sensitivity if the fraction is >3–4%. Further studies will show if increased doses of Xolair® would help also these patients, who seem to represent about 1/3 of the patient population.

S. G. O. Johansson^{1,2}, A. Nopp¹,
H. Öman³, J. Ankerst⁴, L. O. Cardell⁵,
R. Grönneberg⁶, H. Matsols⁷,
S. Rudblad⁸, V. Strand⁹,
G. Stålenheim¹⁰

¹Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute, Stockholm, Sweden; ²Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden; ³MIAB, Uppsala, Sweden; ⁴Department of Medicine, University Hospital, Lund, Sweden; ⁵Department of ENT, Karolinska University Hospital, Stockholm, Sweden; ⁶Department of Medicine, Asthma and Allergy Unit, Karolinska University Hospital, Stockholm, Sweden; ⁷Department of Medicine, Asthma and Allergy Unit, Central Hospital, Falun, Sweden; ⁸Department of ENT, University Hospital, Örebro, Sweden; ⁹Division of Asthma and Allergy, St. Göran Hospital, Stockholm, Sweden; ¹⁰Department of Medicine, Asthma and Allergy Unit, University Hospital, Uppsala, Sweden

Key words: basophils; CD-sens; IgE; omalizumab; responders.

S. G. O. Johansson
Clinical Immunology and Allergy Unit
Karolinska University Hospital L2:04
S-171 76
Stockholm
Sweden

Accepted for publication 7 February 2009

Persistent, allergic asthma has been successfully treated with the anti-IgE antibody omalizumab (Xolair®, Novartis AG, Basel, Switzerland) for several years. The treatment is prescribed on the basis of clinical parameters and the dose adjusted to IgE body pool calculated from pretreatment serum IgE level and body weight (1, 2). However, no adjustments are recommended based on the concentration of disease relevant IgE antibody although the degree of allergen sensitization is related to the relative size of the IgE antibody fraction, i.e. the percentage of IgE antibodies of serum IgE, the latter sometimes referred to as 'total-IgE' (3).

It has been previously reported that the efficacy of Xolair was less in patients with low IgE levels, defined as <75 kU/l (4). When serum samples from a large representative population allergic to cat and house dust mite were analysed for serum levels of IgE antibodies to these allergens in relation to IgE it was found that a significant percentage of the patients with low levels of IgE were IgE-sensitized. Those with the lowest IgE concentrations had the largest IgE antibody fractions (5). It was calculated that, for a hypothetical serum IgE level of 10 kU/l, the threshold recommended for anti-IgE treatment, 25% of the originally mite-and/or cat-sensitized population in the low, <75 kU/l, IgE group would still have significant, i.e.

>0.35 kU_A/l, IgE antibody concentrations in serum. Thus, it seems that, in addition to IgE body pool, not only the presence of IgE antibodies but also the size of the IgE antibody fraction, i.e. the percentage of IgE antibody out of 'total-IgE', should be taken into consideration when calculating the therapeutic dose of anti-IgE.

The aim of this study was to compare the effect of Xolair, given according to recommended dose, in a group of patients IgE-sensitized to cat with a high percentage of IgE antibodies of IgE with a group with a low percentage. The effect of Xolair on IgE-mediated inflammation was measured as basophil allergen threshold sensitivity, CD-sens (3, 6), before and after treatment.

Material and methods

Patients

Patients with allergic rhino-conjunctivitis and/or asthma with IgE antibodies to cat dander were invited to participate in the study. In a screening sample IgE and IgE antibodies to cat were determined and depending on the size of the IgE antibody fraction, i.e. the percentage of cat IgE antibodies of total IgE, patients were recruited either to a low (L) ($\leq 1.0\%$) (18 on active (a) and 10 on placebo (p)) or a high (H) ($\geq 3.8\%$) (20 on active (a) and 11 on placebo (p)) percentage group, and randomly allocated to active or placebo. These population fractions represent the lower and upper third of a nonselected population of patients IgE-sensitized to cat (5). All patients were drug free and had not previously been subject to allergen specific immunotherapy to cat.

Study design

Anti-IgE (Xolair®, Novartis, Switzerland) was given for 16 weeks at a dosage based on serum IgE concentration and patient body weight as recommended by the manufacturer (2). Immediately before the first dose blood samples were drawn for determination of CD-sens ('before') and IgE- and IgG4-antibodies to cat allergen. Blood samples for antibody and CD-sens assays ('after') were drawn at the time for the first missing injection after the 16 week period, i.e. 20 weeks after the first injection for those receiving monthly injections and 18 weeks for those receiving bi-weekly injection.

At the last visit, the patients were asked if their allergy, during the study, had been better, unchanged or worse. No other clinical evaluation was performed.

The study was accepted by the Local Ethics Committee at Karolinska Institute, Stockholm, Sweden and by the Swedish Medical Products Agency (EudraCTnr: 2006-002771-40).

Calculation of CD-sens

Basophil allergen threshold sensitivity, CD-sens, was analysed by flow cytometry after incubation with serial dilutions of cat allergen extract and defined as the inverted value for the allergen concentration giving a 50% of maximum CD63 up-regulation multiplied by 100 (3, 6). The intra assay coefficient of variation, CV, was 13.1% and the inter assay CV 5.4% (6). There is a significant correlation between CD-sens, skin prick test titration and nasal provocation allergen sensitivity (3). Percentage CD63 up-regulation,

after stimulation with one or a few high doses of the cat allergen, was taken as a measure of basophil reactivity (6).

Calculation of allergen binding activity

The CD-sens from untreated whole blood was compared to the CD-sens in blood samples where the plasma had been removed before allergen stimulation. The ratio CD-sens after/before washing was calculated and termed 'allergen binding activity' (ABA) (7).

Calculation of IgE and FcεRI receptors

The number of IgE molecules and FcεRI receptors per basophil was calculated using a FITC-conjugated antibody to IgE (Dako Cytomation, Glostrup, Denmark) or FcεRI (eBioscience, San Diego, CA, USA) and compared to a standard curve of Calibration Beads (Dako Cytomation) arbitrarily multiplied by 10. With assigned molecules of equivalent fluorochrome (MEF) values for the fluorescent bead populations, arbitrary units of mean fluorescence intensity (MFI) could be transformed into absolute units.

Immunoglobulin analyses

The serum concentrations of IgE (kU/l) were determined by ImmunoCAP® Total IgE (Phadia AB, Uppsala, Sweden), and IgE-(kU_A/l) and IgG4-antibodies (mg/l) to cat (e1) by ImmunoCAP® Specific IgE (Phadia AB) according to the manufacturer's instructions.

Statistical analyses

For comparisons between groups a difference in medians or proportions of negative results was tested with the chi-square test with Yates correction. A change within a group (before–after) was tested with pair wise *t*-test or sign test. A *P*-value of <0.05 was considered as statistically significant. For each group the median and the range are presented.

Results

Allergen sensitivity

In the group with a high percentage of disease relevant IgE antibodies to cat (group H) 31 patients completed the study. Of those, 20 received active drug (Ha) and 11 placebo (Hp). The corresponding figures for those with a low percentage (group L) were 18 active (La) and 10 placebo (Lp) patients. There were no significant pretreatment differences in IgE antibody percentages (Table 1) or CD-sens (Table 1; Fig. 1) between Ha and Hp or between La and Lp.

During treatment there was a significant drop in CD-sens from before to after in both active groups ($P < 0.001$) but not in the placebo groups (Table 1). The before/after ratio was significantly ($P < 0.05$) lower in the Ha-group compared to the La-group.

After treatment, the La-group had significantly ($P < 0.05$) lower CD-sens (<0.01 ; <0.01 –2.3) than the Lp-group (0.9; <0.01 –4.4), while this difference was not

Table 1. CD-sens and percentage of IgE antibodies to cat before (Before) and after (After) treatment with active (a) or placebo (p), presented as median and range, in the high (H) and low (L) percentage group

Group	No. of patients	% IgE-ab	CD-sens Before	CD-sens After
Ha	20	6.9 (4.0–21.9)	15.4 (1.3–72.0)	1.8 (0.2–8.0)
Hp	11	6.0 (3.9–17.8)	27.7 (0.4–60.0)	15.6 (0.5–145)
La	18	0.5 (0.2–1.1)	0.9 (0.05–16.0)	<0.01 (<0.01–2.3)
Lp	10	0.4 (0.1–1.0)	1.3 (0.03–6.0)	0.9 (<0.01–4.4)

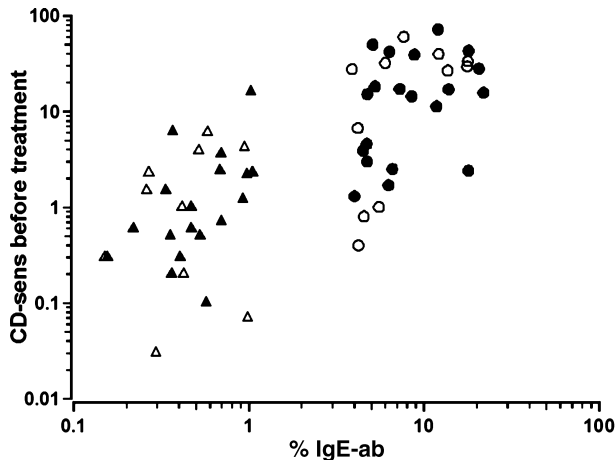


Figure 1. The distribution of CD-sens in relation to percentage of IgE antibodies to cat before start of trial in the high group receiving active (●) or placebo (○) compared to the low group on active (▲) or placebo (△).

significant ($P < 0.06$) in the H-group (Table 1). In the La-group, 13 of 18 patients had a negative (<0.01) CD-sens after treatment, while in the Ha-group, no patient's CD-sens had become negative (Fig. 2). The higher the IgE, the more responders were found (Fig. 3). No patient in the Hp-group became CD-sens negative. In contrast, two patients in the Lp-group did; however, their CD-sens before were very low, i.e. <0.1 .

CD-sens before was significantly higher ($P < 0.001$) in the Ha-group than the La-group. However, in the before CD-sens 1–10 interval (overlapping the low and the high group) six of the eight La-patients but none of the seven Ha-patient developed a negative CD-sens after.

There was a significant decrease in the basophil reactivity during treatment both in the Ha- and La-groups ($P < 0.01$), but not in the placebo groups. However, five patients in the Ha- and two in the La-group increased their reactivity (range 12.7–26.1% and 72.6–86.4%, respectively). Similarly, in the placebo groups, three patients in the Hp-group (range 3.7–19.3%) and five in the Lp-group (range 12.0–131%) increased their reactivity.

There were no differences between active and placebo treatment in the patients' report on their allergy during the 16 weeks of the trial.

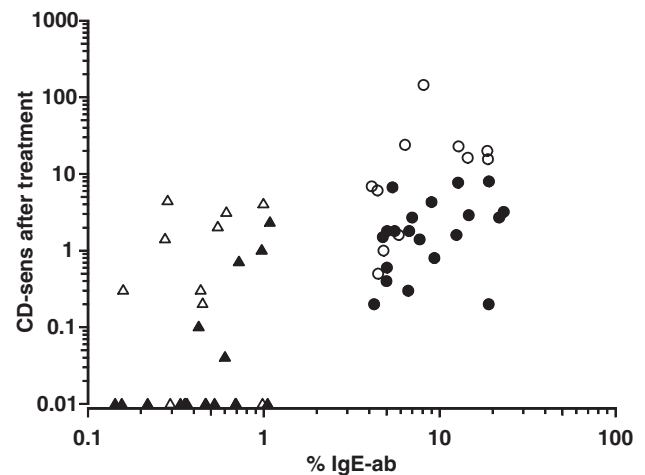


Figure 2. CD-sens after treatment in relation to percentage of IgE antibodies to cat before start of trial in the high group receiving active (●) or placebo (○) compared to the low group on active (▲) or placebo (△).

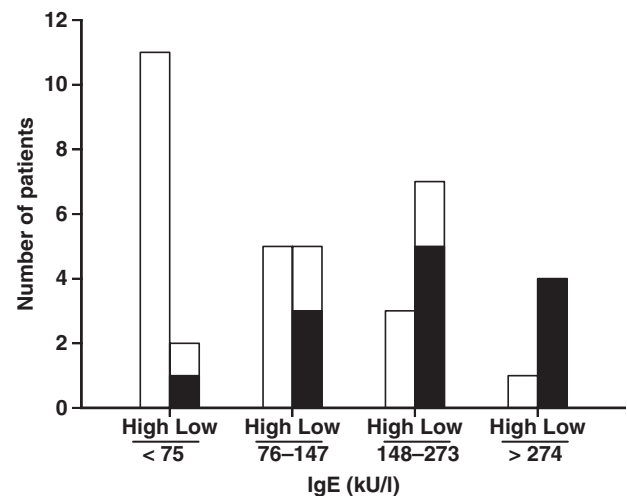


Figure 3. Number of CD-sens positive (□) and negative (■) patients in the high and low group after treatment with Xolair in relation to serum IgE concentration before trial.

A significant ($P < 0.05$) decrease in ABA during therapy was seen in the Ha-group, while the median IgG4 antibody concentration increased significantly ($P < 0.05$) in both the La- and Lp-group. No other significant changes for ABA or serum IgG4 antibody values were seen.

IgE and FcεRI on basophils

There was no essential difference in number of IgE molecules on the basophils in the four groups before treatment (Table 2). However, the medians of the before/after ratios, 11.0 and 12.3, respectively, were significantly

Table 2. The change in number of IgE molecules and FcεRI per basophil (numbers divided by 1000) and IgE-stained cell population distribution, median and range, before (Before) and after (After) active (a) or placebo (p) treatment in the high (H) and low (L) percentage groups. Population 1 is the basophils appearing bright in the flow cytometer histogram

Group	IgE-mol per cell Before	IgE-mol per cell After	FcεRI per cell Before	FcεRI per cell After	Percent IgE positive cells Population 1 Before	Percent IgE positive cells Population 1 After
Ha	473 (66–642)	40 (11–145)	233 (52–336)	23 (13–61)	96 (25–99)	5 (2–29)
Hp	499 (195–860)	494 (154–678)	331 (120–365)	295 (104–366)	97 (72–99)	96 (68–98)
La	505 (240–849)	40 (11–146)	293 (170–814)	27 (6–90)	96 (91–100)	9 (1–56)
Lp	568 (447–750)	620 (433–804)	287 (242–387)	283 (249–373)	97 (82–99)	96 (91–100)

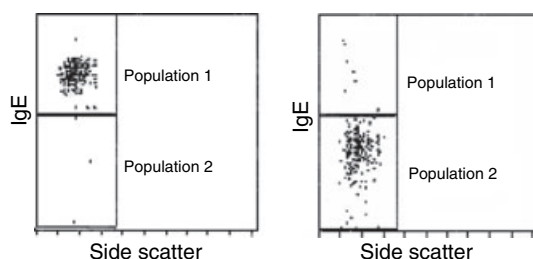


Figure 4. The distribution of IgE-stained basophils analyzed with flow cytometry. The left histogram shows an example of the distribution of IgE on basophils before treatment and the right histogram shows the distribution after treatment with Xolair.

($P < 0.001$) higher, and the medians of the after treatment number of molecules were significantly ($P < 0.001$) lower in the Ha- and La-groups, compared with placebo. However, there were no differences between the Ha- and the La-groups.

From the distribution in the flow cytometer a bright (Population 1) and a dim (Population 2) population of IgE-stained basophils could be identified (Fig. 4). During the study the percentage stained cells in Population 1 in the Ha-group dropped significantly, $P < 0.001$, (from median 96–5%) as compared to the Hp-group (97–96%) (Table 2). Almost identical and significant ($P < 0.001$) changes were seen in the La-group (from median 96–9%) compared to the Lp-group (97–96%).

Similar results were found also for the IgE receptor, FcεRI; the ratio before/after was significantly ($P < 0.001$) higher in both the Ha- and La-groups compared to placebo (Table 2).

Discussion

Most classical allergic diseases e.g. allergic asthma, rhinoconjunctivitis, food allergy and allergic anaphylaxis are caused by an IgE antibody mediated inflammation (8, 9). Thus, elimination of IgE would be effective treatment, irrespective of target organs involved or allergen triggering the reaction. However, it is obvious that if IgE

molecules are targeted, the concentrations of the pathogenic IgE antibody molecules must be reduced to levels that can not trigger the key cells in allergic inflammation, i.e. mast cells and basophils.

Xolair® was registered by the FDA in 2002 and by the European Union drug authorities in 2005 for treatment of severe allergic, IgE-mediated, asthma, and the diagnosis should be based on a documentation of the allergy by skin testing or determination of IgE antibodies to relevant allergens (2). There is a strict quantitative relation between the amount of Xolair® injected and the size of the IgE body pool, i.e. serum IgE concentration and body weight. The recommended doses aim to decrease free IgE in circulation to approx. 10 kU/l by forming IgE-Xolair immune complexes which are then eliminated by the immune system with a $T_{1/2}$ of approx 3 weeks. It is expected, but not recommended for monitoring, that when this steady-state is reached there will be no IgE antibodies present that can trigger an allergic reaction.

If the pretreatment evaluation is based only on skin tests, no information is obtained about the amount of IgE antibodies that needs to be eliminated. The skin test, like allergen challenge of bronchial or nasal airways, is dependent on target organ mediator sensitivity, which is not necessarily correlated to IgE antibody concentration. In addition, the error of the method does not permit quantitative evaluation; for allergen sensitivity measured by skin tests a CV of 100–200% has been reported (3). It seems logical that, if the treatment is aimed at eliminating disease relevant IgE antibodies, the amount of IgE antibodies should be determined (10). Consequently, tests such as skin tests and allergen challenge tests are not useful for evaluation of treatment efficiency while basophil allergen threshold sensitivity (CD-sens) assays (3) seem to be most valuable.

When the efficacy of Xolair® was compared to placebo in patients that had participated in clinical trials of the drug and the patients were grouped according to serum IgE levels, it was found that there was a significant effect in patients with higher serum IgE levels, but not with low serum IgE levels, i.e. IgE 30–75 kU/l (4).

We have shown that the size of the serum IgE antibody fraction, i.e. the percentage of disease relevant IgE

antibodies of 'total IgE', increases with decreasing IgE levels (5). Thus, in a population of cat- and house-dust mite allergic patients, high median percentages of IgE antibodies (3.2–3.7%) were found in the low, (30–75 kU/l) IgE group, and the reverse (0.3–1.3%) in the high (150–700 kU/l) group. These findings stimulated a hypothesis that the currently recommended doses of Xolair® were not effective in patients with a high percentage of IgE antibodies out of their IgE. The hypothesis was evaluated in this study, in which the effect of Xolair® in recommended doses was tested in 31 cat-allergic patients with a cat-specific IgE antibody percentage corresponding to the upper third of our cat allergic population and compared to 28 patients from the lower third. According to a double-blind placebo controlled protocol 20 (high group) and 18 (low group) patients were receiving Xolair® and 11 and 10, respectively, were receiving placebo. The effect of Xolair® was determined as changes in basophil allergen threshold sensitivity, CD-sens. This approach is, like Xolair®, specifically directed to the IgE link in the allergic chain reaction starting with allergen exposure and ending up with symptoms of allergic disease. CD-sens as a surrogate marker for clinical allergen sensitivity has been evaluated and has been found to correlate with skin prick test titer (3), nasal allergen challenge (3) and bronchial allergen challenge (unpublished data). To further document the usefulness of CD-sens as a mirror of clinical allergen sensitivity more studies regarding correlations with bronchial allergen challenge as well as food allergen challenge are under evaluation.

As expected, in Xolair treated patients the numbers of IgE molecules and FcεRI on basophils were significantly decreased in both the high and low group treated with active substance as opposed to patients treated with placebo.

There was also a significant decrease in CD-sens in both groups treated with active drug in contrast to placebo. However, no patient in the high group turned negative, compared with 13/18 in the low group. As in the study of Xolair efficacy in relation to serum IgE levels (4) most of the responders had moderate to high serum IgE levels. The lack of effect in the high percentage group might, to a certain degree, be explained by the fact that this group had a significantly higher CD-sens before treatment than the low group. However, a small number of patients, small because they were selected to be as different as possible, in the two groups had a CD-sens in the same range (CD-sens 1–10) before treatment. All patients from the low percentage group became negative on active drug, but none from the high group.

Allergen stimulation, e.g. during the initiation phase of ASIT, results in a decrease in CD-sens probably due to an increase in plasma ABA, e.g. IgG4 antibodies (11). However, in another study, we analysed blood from patients that had received Xolair-treatment for 6 years and followed them for a year after withdrawal. Many had nonreacting basophils (7) but this could not be attributed

to ABA or IgG4 antibodies. In the present study, no consistent effect was seen on ABA or cat IgG4 antibody levels. Probably the possible allergen exposure was too small.

Five patients in the low group receiving active drug did not become CD-sens negative after treatment. For three of them, the before/after ratio was rather small, 1.2–2.6, which could indicate an inefficient response of the basophils. However, all three patients responded well immuno-chemically to treatment as measured by decreased amounts of IgE and FcεRI on their basophils. One possible explanation could be that these patients have very sensitive and unstable basophils that may be triggered by much lower numbers of IgE cross-linkings, as compared to the 'normal' approx. 1000 bridges reported (12). Another possibility is *in vivo* allergen stimulation. We have in earlier studies seen that birch- and timothy-pollen allergic patients on Xolair® can get an increase in CD-sens to those pollens during the respective pollen season (unpublished observation). The most likely explanation of this increase is that even a partial symptom cover by Xolair® allows the patient to expose himself to disease relevant allergens. In this study, it is possible that these three patients did expose themselves to cats to such a degree that the IgE system responded with a booster compensating for the Xolair® induced reduction in CD-sens.

Although the median basophil cell reactivity decreased in both of the active treatment groups, but not the placebo groups, indicating an effect of anti-IgE, quite a large proportion of the patients instead had a remarkable increase (approx. 10–90%). Similar, or even larger, increases were found in the two placebo treated groups. Interestingly, in the study of Xolair® withdrawal after 6 years of treatment (8), no relation was seen between any change in basophil reactivity and clinical or immunological parameters.

No attempts were made to objectively evaluate the effect of active drug or placebo on symptoms. After the 16 weeks trial the patients were asked if their symptoms had been worse, unchanged or better during the trial. No difference between actively or placebo treated patients was found. Perhaps the patients avoided cat contact as they were used to. However, the study was performed during the fall and winter seasons and it is most likely that factors like common infections confounded the evaluation.

In conclusion, Xolair® actively prohibits allergen-initiation of an IgE antibody triggered allergic inflammation by eliminating IgE. Thus, the patient's allergic symptoms must be well documented to be due to IgE antibodies before starting treatment with Xolair and this can not be accurately performed by skin testing and case history. The presently recommended dosing for Xolair® seem to be effective only in cases with a small fraction, in this study < 1%, of disease-relevant IgE antibodies out of 'total IgE'. If the fraction is larger, i.e. > 3–4%, a higher dose must be

considered. When calculating the disease relevant IgE antibody fraction, antibodies specific to noncross reacting allergens, to which the patient is simultaneously exposed, must be added (13). Clinical trials, including detailed monitoring of clinical symptoms, will further document our recommendations for treatment efficacy.

Acknowledgments

We would like to thank Ingegerd Ågren-Andersson for performing the ImmunoCAP® analyses and Åse Olerud for helping with the basophil analyses. The statistical analyses were performed by Göran Nilsson. Xolair and placebo was kindly provided by Novartis Horsham, UK.

References

1. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson BG, Chung KF et al. Efficacy and safety of a recombinant anti-IgE antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;**34**:632–638.
2. Xolair (omalizumab), Summary of Product Characteristics: <http://www.emea.eu.int/humandocs/PDFs/EPAR/Xolair/H-606-PI-en.pdf>.
3. Nopp A, Johansson SGO, Ankerst J, Bylin G, Cardell LO, Grönneberg R et al. Basophil allergen threshold sensitivity. A useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006;**61**:298–302.
4. Holgate ST. Implications for clinical practice, in anti-IgE therapy. A new area in the control of severe persistent allergic asthma? European Respiratory Society Congress, Copenhagen, 19 September, 2005.
5. Johansson SGO, Öman H, Nopp A, Pettersson S. The importance of IgE antibody levels in anti-IgE treatment. *Allergy* 2006;**61**:1216–1219.
6. Johansson SGO, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L et al. Passive sensitization by blood transfusion. *Allergy* 2005;**60**:1192–1199.
7. Nopp A, Johansson SGO, Ankerst J, Palmqvist M, Öman H. CD-sens and clinical changes during withdrawal of Xolair after 6 years of treatment. *Allergy* 2007;**62**:1175–1181.
8. Johansson SGO, Hourihane JO'B, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
9. Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;**113**:832–836.
10. Rolinck-Werninghaus C, Keil T, Kopp M, Zielen S, Schauer U, von berg A et al. Specific IgE serum concentrations is associated with symptom severity in children with seasonal allergic rhinitis. *Allergy* 2008;**63**:1339–1344.
11. Nopp A, Cardell LO, Johansson SGO, Öman H. CD-sens, a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009;**64**:811–814.
12. MacGlashan DW, Bochner B, Adelman D, Jardieu P, Togias A, McKenzie-White J et al. Down-regulation of FcεRI expression on human basophils during *in vivo* treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;**158**:1438–1445.
13. Nopp A, Johansson SGO, Lundberg M, Öman H. Simultaneous exposure to several allergens has an additive effect on multisensitized basophils. *Allergy* 2006;**61**:1366–1368.